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By: _____

PATENT
Attorney Docket No.: 002558-065010US
Client Ref. No.: BRP00122

10/W.M. 9/4/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

CORY M. PANATTONI and LEE OLECH

Application No.: 09/973,179

Filed: October 5, 2001

For: COATING OF PRE-CAST ELECTROPHORESIS SLAB GELS

Customer No.: 20350

Confirmation No.: 4474

Examiner: Olsen, Kaj K.

Technology Center/Art Unit: 1753

DECLARATION OF CORY M. PANATTONI UNDER 37 CFR § 1.132

Mail Stop Non-Fee Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, CORY M. PANATTONI, hereby declare as follows:

The experiments that are described below and whose results are shown in the attached exhibits were performed either by me or under my direction and supervision:

A series of electrophoretic separations of standard protein mixtures were performed on pre-cast vertical slab-shaped polyacrylamide gels that had been cast between flat plates of a polystyrene-acrylonitrile blend, the interior surfaces of the plates having been coated

with various resins. Each pre-cast gel was 1 mm in thickness and 7 cm in height and contained the buffer TRIS-HCl (tris(hydroxymethyl)aminomethane hydrochloride) in a concentration gradient extending from 4% by weight at the top edge of the gel to 20% by weight at the bottom edge. Each gel had been stored at 4°C for twelve weeks prior to its use in the electrophoretic separation. Three mixtures were separated in separate lanes on each gel, each mixture repeated in 3 to 8 lanes per gel. The three mixtures were: (1) Biotinylated SDS-PAGE Protein Standards Broad Range (a product of Bio-Rad Laboratories, Inc, assignee of the above-referenced patent application), (2) Precision Plus Protein Standards (a product of Bio-Rad Laboratories, Inc.), and an E. coli lysate. Separations were conducted with a constant voltage of 200 V over a period of 54 minutes.

Four separate experiments were performed, each one differing from the others by the coating on the interior surface of the plastic plate. As received, all plates were initially coated with polyvinylidene chloride (PVDC), and one experiment utilized the PVDC-coated plate as the control. In each of the three other experiments, a coating was applied over the PVDC coating. These outer coatings were polyvinyl pyrrolidone (PVP) of molecular weight 40,000, polyethylene glycol (PEG) of molecular weight 20,000, and polyvinyl alcohol (PVA) of molecular weight 14,000 (all are weight-average molecular weights). All three outer coatings are water-soluble polymers and were applied as 1% by weight aqueous solutions.

The gels after the separations were performed are shown in the four attached exhibits as follows:

Exhibit 1: coating in contact with gel: PVDC (control)

Exhibit 2: coating in contact with gel: PVP

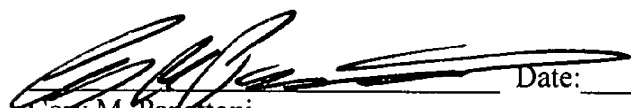
Exhibit 3: coating in contact with gel: PEG

Exhibit 4: coating in contact with gel: PVA

While samples of the three protein mixtures do not occupy the same lanes in each of the gels, the first three lanes (starting from the left) on each of the gels (and other lanes with the same band distribution) are separations of the Biotinylated SDS-PAGE Protein Standards Broad Range. The next lanes with a similar number of bands but a different distribution than the first three (and other lanes with the same band distribution) are separations of the Precision Plus Protein Standards, and the darker lanes each containing a larger number of bands closer together are separations of the E. coli lysate.

The control (PVDC-coated cassette walls, Exhibit 1) shows shadow bands (i.e., shadows below the dark lines) in separations of each of the three protein mixtures. The PVP-coated cassette walls (Exhibit 2) show a lessening of the shadow bands, particularly in the mid-range to faster-migrating bands (toward the bottom end of the separations). The PEG-coated cassette walls (Exhibit 3) show further removal of the shadow bands, and the PVA-coated cassette walls (Exhibit 4) show essentially complete elimination of the shadow bands.

I further declare that all statements made herein are true and that all statements made on information and belief are believed to be true, and that I have been warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.



Date: 8/7/03

Cory M. Panattoni

Attachments
MHH:mhh
60006006 v1